UNDERTAKING GENETIC MAPPING OF SUGARCANE SMUT RESISTANCE

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Abstract

Smut is one of the most important diseases of sugarcane and has a worldwide distribution. It can cause severe yield losses when a susceptible variety is grown in a smut infested area. Resistance is therefore a major concern for most sugarcane breeding centers.

A study on the genetic determinism underlying sugarcane smut resistance was initiated. A genetic mapping strategy was chosen that focused on a cross between cultivar R 570 (resistant) and cultivar MQ 76/53 (highly susceptible) which showed a segregation for smut resistance in a preliminary field trial. An AFLP map is being constructed for both parents of the cross. At the same time, field trials and greenhouse experiments have begun using different artificial inoculation methods to assess the resistance of 200 individual progeny.

This paper presents first results on smut occurrence among this progeny and correlations between segregating markers and resistance to smut. Other characters have also been observed (Brix, number of stalks, rust resistance). The possibility of identifying the different components involved in smut resistance and the interest of locus specific markers (SSR, resistance gene analogs, etc) to refine the genetic map are discussed.

Keywords: sugarcane, smut, Ustilago, QTL, disease resistance, AFLP

Introduction

Smut caused by *Ustilago scitaminea* is one of the most important diseases of sugarcane. It is transmitted over long distances by wind-blown spores and therefore has a world-wide geographical distribution (Comstock, 2000). Smut is a major concern for breeders and various screening tests and rating protocols have been developed in order to assess the resistance of new varieties (Walker,1987). Quantitative genetic studies have indicated that narrow-sense heritability for this character is moderate (Wu *et al*., 1983; Chao *et al*., 1990). Nevertheless, little is known about the genetic control of smut resistance.

QTL mapping is a powerful tool to address the dissection of complex disease resistance into simple mendelian components (Young, 1996). In highly polyploid sugarcane interspecific hybrids, the prerequisite for QTL mapping is to achieve a good coverage of the genome. The amplified fragment length polymorphism (AFLP) technique is suitable for this purpose on account of the numerous markers it generates. An AFLP genetic map of the Reunionese cultivar R 570 has been constructed, using a selfed progeny of R 570 (Hoarau *et al*., 2001). This map covers about half of the genome. A major rust resistance gene has already been tagged in this cultivar (Daugrois *et al*., 1996, Asnaghi *et al*. 2000). The challenge is now to understand more complex characters such as smut resistance in order to facilitate their manipulation.

This paper reports preliminary results of a study designed to map smut resistance components in a bi-parental cross between cultivar R 570 and cultivar MQ 76/53. We present the first data obtained from agronomic trials undertaken in Reunion island and AFLP genotyping.

Material and methods

Field trials

Two bi-parental crosses involving the resistant cultivar R 570 (H 32/8560 x R445) were studied : NCo376 x R 570 and R570 x MQ 76/53. NCo376 (Co 421 x Co 312) is a moderately susceptible cultivar and MQ76/53 (Trojan x SES 528) is a highly susceptible one. R 570 was used as a female after emasculation by hot water treatment in the second cross. R 570 and NCo376 are present-day cultivars while MQ 76/53 is an F1 interspecific hybrid between a cultivar and a *S. spontaneum* clone. For each progeny, a preliminary experiment with 100 clones was planted in November 1998 at CERF-le Gol station using a randomized block design with two replicates. The basic plot consisted of a 2.5 m row planted with eight inoculated cuttings. The aim of these preliminary trials was to determine which of the two families had the most suitable smut segregation for a QTL analysis and thus should be used for genotyping and further agronomic trials.

Sugarcane was inoculated with smut by dipping three bud cuttings for 20 minutes in a spore suspension of $5x10^6$ spores per ml. Viability of spores was previously checked on agarose in Petri dishes. Inoculated cuttings were incubated for 24 hours under high humidity before planting. Smut incidence was measured by counting newly emerged whips every 15 days (and the number of whips (NW) was summed over the entire crop cycle). Old whips were marked with paint to avoid counting them twice. This assessment was performed in plant cane and in the first ratoon. This trial will be kept under investigation until the end of the second ratoon. Besides smut evalutation, advantage was taken of the present trial to measure other characters in the first ratoon: resistance to rust caused by *Puccinia melanocephala*,

number of millable stalks and brix. Rust resistance was assessed according to a 1-9 scale (Tai *et al*, 1981). Brix was measured with a hand refractometer using six individual millable stalks randomly chosen per plot. The number of millable stalks was counted over the entire plot.

AFLP genotyping

Eleven AFLP primer combinations (GIBCO BRL AFLP Kit genome I) were used to produce a first set of polymorphic markers with the R 570 x MQ 76/53 progeny. Among these 11 primer combinations, nine had already been used for R 570 AFLP map (Hoarau *et al*, 2001). Therefore, most of the bands specifically inherited from R 570 in the biparental population, could be labelled in accordance with the name already defined in the published map.

Statistical analysis

General statistics were calculated using the UNIVARIATE procedure (SAS, 1990). Normality was rejected if the Shapiro and Wilk probability was less than 0.10. Broad sense heritability was calculated at the experimental design level as follows:

$$H_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + (\sigma_e^2 / 2)}$$

Genetic (σ_g^2) and error (σ_e^2) variance estimates were obtained with SAS VARCOMP procedure.

Marker segregation was compared with expected ratios (1:1 for simplex markers, 3:1 for duplex and 7:1 for triplex). A χ^2 test was performed at the significance level of P=0.05.

Marker-trait linkages were detected by the Kruskall-Wallis rank test. This analysis was performed with mapqtl software (Van Ooijen *et al*, 1992). Only associations significant at P<0.01 were retained.

Results

Choosing the best population

As shown in Figure 1, the cross between R 570 and MQ 76/53 appeared more susceptible on average than the cross between NCo376 and R 570. This is particularly true in first ratoon (2000) where smut incidence increased significantly in the first cross but remained low for the second cross. This is in agreement with the level of susceptibility observed under local Reunion conditions where MQ 76/53 is known to be highly susceptible and NCo376 moderately susceptible. The cross R 570 x MQ 76/53 presented a wider segregation and should be more appropriate for the detection of QTLs involved in smut resistance. The distribution of the number of whips counted in first ratoon is presented in Figure 2. It ranges from clones with no whips to a few highly infected clones. Therefore we decided to focus on the R570 x MQ 76/53 cross for AFLP genotyping and further field trials. Thus, the results presented below only concern this cross.

Quantitative trait analysis

Stalk number per plot was the only parameter that was normally distributed (Table 1). Heritability of rust resistance was very high and heritability of 'refractometer Brix' was fairly high considering the low number of replications. Heritabilities of number of whips (NW) and number of stalks (SN) were moderate in this preliminary trial and could probably be improved with a more powerful experimental design. The derived ratio NW/SN showed a low heritability. For rust resistance there was a clear 7 resistant: 1 susceptible segregation ratio ($P>c^2=0.65$), based on presence/absence of sporulating pustules. This suggested that three putative major genes for rust resistance may segregate. Since it is known that R 570 contains a single major gene (Daugrois *et al*, 1996), it can be postulated that MQ 76/53 may carry two genes.

Production of a first set of AFLP markers

A total of 340 scorable polymorphic markers were produced using 11 primer combinations of which 183 (54%) are simplex markers (with a 1:1 presence versus absence segregation ratio) (Table 2). Only 25% of the polymorphic markers were common between the two parents, while 75% were specific to one of the two parents. These proportions are similar to those observed in another biparental population (Offmann, [1] unpublished data). Only simplex markers will be used in the future to build the parent maps. This first set of markers encompass 123 markers already mapped in R570 by Hoareau *et al* (2001) which are scattered on 66 of the 120 cosegregation groups of the reference map.

First attempt to detect marker-trait associations

Despite the limited progeny size investigated to date, a first detection of marker-trait associations was attempted for each measured character (340 markers x 5 traits resulting in 1700 Kruskal-Wallis tests). A total of 16 associations were found at P=0.01, among which four were significant at P=0.005 and one at P=0.001 (Table 3). The exact significance of these results remained to be clarified when (i) data is available on a larger progeny (ii) independance of markers is established after genetic mapping. One marker was found to be linked at P=0.005 to smut resistance, measured as the number of whips (NW) produced per plot. It is a simplex marker of resistance inherited from R 570. Also detected were two markers common between the two parents at P=0.01. All these three associations were detected for the derived ratio NW/NS. Two markers were detected for the number of stalk (NS). They have a negative effect on the trait even though they originate from cultivar MQ 76/53 which is characterised by a high tillering ability. Three markers were detected for refractometer Brix, among which two were simplex markers derived from MQ 76/53. These markers were associated with nearly one point increase of Brix. For rust resistance, five marker-trait associations were identified; four markers are specifically derived from R 570 and one is contributed by MQ 76/53. One marker (aaccac_r6) is known to be located at 2.5 cM from the major resistance gene of cultivar R570 (Asnaghi, 2000). The effect of this marker may be diluted because of the existence of two other putative major genes from MQ 76/53, as postulated above.

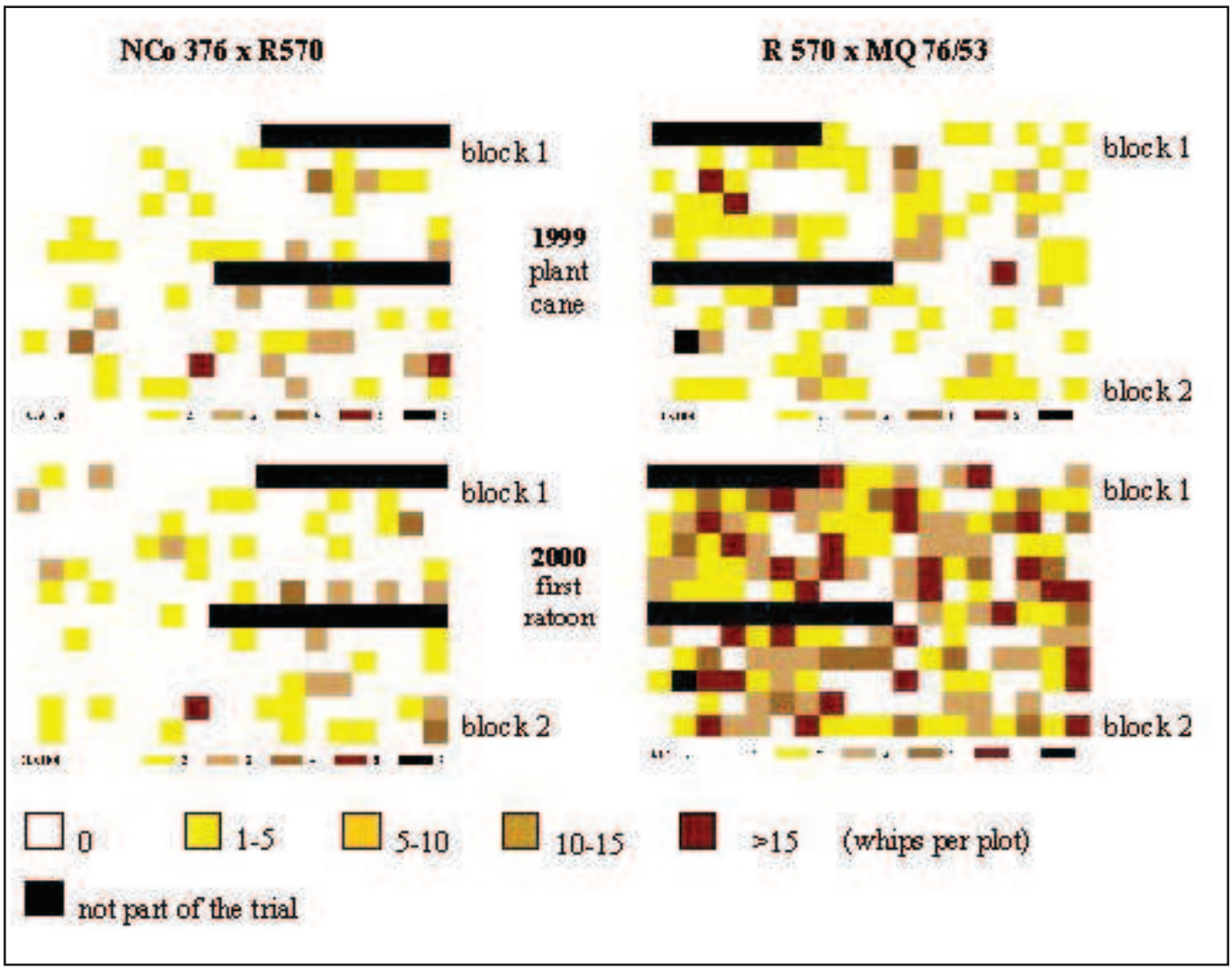

Figure 1. Cartography of smut incidence (number of whips per plot) in the two biparental crosses assessed for two crop cycles. Each plot is represented by an individual square. The intensity of colour is related to smut incidence (GMAP procedure, SAS).

Future development of this project

This study is still in progress. Data presented here are only partial and no definitive conclusions can be drawn. Nevertheless, these preliminary data show that the focus is on a very interesting cross which presents a wide segregation for smut resistance but also for other important agronomical characters. This is a consequence of the contrast between the two varieties.

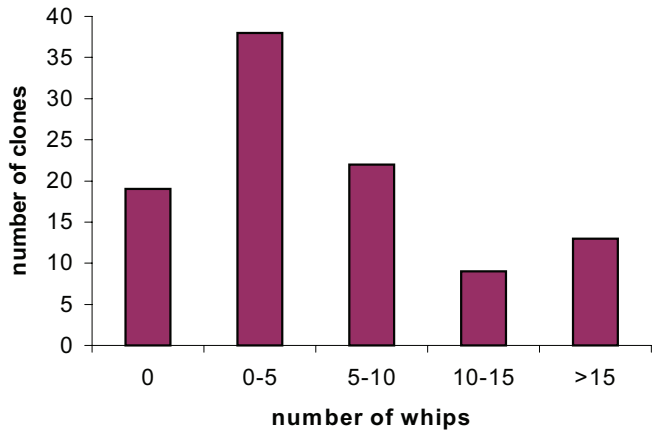

Figure 2. Distribution of the number of whips per plot in first ratoon among 100 progeny in the cross R 570 x MQ 76/53.

In order to effectively dissect smut resistance components in the material, work will have to be done on a larger scale. To this end, it is intended to invest efforts on the following points :

1) Improvement of smut resistance characterisation

Two artificially inoculated trials involving more progeny (200 clones instead of 100) of the R 570 x MQ 76/53 cross, one of which is already planted, will provide a better evaluation of clones genotypes regarding their resistance to smut. Moreover, these replicated trials will include regularly inserted spreader rows of highly susceptible parent MQ 76/53. An effort will also be made to characterise the different mechanisms involved in smut resistance by conducting greenhouse trials. For that purpose, various inoculation techniques will be used, some of which will be designed to bypass the resistance barrier of the bud scale (for example the bud puncture method and injection of young shoots).

Table 1. General statistics for the traits studied in the R 570 x MQ 76/53 cross.

Traits	Mean	standard deviation	Min	Max	Normality[1]		Heritability[2]
Number of Whip (NW)	7.6	9.9	0	60	0.0001	rejected	0.64
Stalk Number (SN)	33.685	9.06	16	59.5	0.1389	accepted	0.63
Ratio (NW/SN)	24.24	35.84	0	231	0.0001	rejected	0.29
Brix	21.06	1.39	16.17	23.58	0.0016	rejected	0.81
Rust	1.26	0.67	1	5.5	0.0001	rejected	0.97

Normality1: Pr<W from Shapiro and Wilk test performed on the mean values for two replications

Heritability2: broad sense heritabilities at the experimental design level

Table 2. Type of AFLP polymorphism produced.

Origin of markers	Polymorphic markers	Number of markers per type in R 570 x MQ 76/53				Comparison with Hoarau's map (from R 570 selfed)	
		Simplex	Duplex	Triplex	Other	Common markers (/939)	Nb of CG2 covered (/120)
R570	124	86	10	0	28	73	46
MQ 76/53	132	97	15	3	17	0	0
Common	84	0	39	26	19	50	41
Total	340	183	64	29	64	123	66

1 Segregation tested with a Chi2 test at P=0.05. Skewed markers and multiplex markers(>triplex) are shown in the 'other' column

2 CG: cosegregation group

2) Intensive production of markers in order to cover two genomes

It is noticeable that 11 AFLP primer pairs only produced around one hundred simplex markers for each variety in comparison with the 884 markers (from 37 primers pairs) used to build the reference AFLP map (Hoarau *et al*, 2001). To obtain the same genome coverage it will be necessary to use at least twice the number of AFLP primer pairs than those that were used to build the reference map. This is due to the fact that work is being done with a biparental cross instead of a selfed progeny as for the reference map.

Locus specific markers must also be used in order to assign cosegregation groups to their respective linkage group. Microsatellites, now available for sugarcane, will be very helpful. These markers should provide a framework that will facilitate comparison between maps and QTL detections studies. Detected quantitative alleles might then be gathered into a particular locus.

3) Utilisation of candidate markers

New opportunities in the aim to understand the genetic determinism of smut resistance and disease resistance in general are expected with the Brazilian sugarcane EST program (SUCEST). This program should soon provide the scientific community with interesting sequences of genes involved in plant defense mechanism (Kuramae *et al*, 2001) that could be used as candidate markers.

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Table 3. Significant marker-trait associations detected in R 570 x MQ 76/53 progeny at P<0,01 according to the Kruskall-Wallis mean-rank test.

	Hoarau's map	segregation		number of whips (NW)			stalk number (SN)			ratio NW/SN			rust resistance			Brix		
markers	CG[3]	n(a)[4]	n(b)[4]	sig[1]	mean(a)[2]	mean(b)[2]	sig[1]	mean(a)[2]	mean(b)[2]	sig[1]	mean(a)[2]	mean(b)[2]	sig[1]	mean(a)[2]	mean(b)[2]	sig[1]	mean(a)[2]	mean(b)[2]
R 570 specific																		
aagctg_r4		47	50	****	10.13	5.38				****	34.49	14.74						
aacctg_r10		40	58										***	1.07	1.40			
actcag_rx2		6	92										***	1.63	1.24			
accctc_r4	120	46	47										***	1.08	1.42			
aaccac_r6	60	59	39										****	1.42	1.02			
MQ 76/53 specific																		
actcat_m14		52	46										***	1.41	1.10			
actcat_m3		54	43				***	35.97	31.03									
accctc_m8		17	77				****	39.85	32.32									
actcac_m14		47	50													****	20.64	21.48
actcac_m2		46	44													*****	20.64	21.62
common																		
aagctg_rm5		27	69	***	13.37	5.51				***	43.85	16.87						
acacta_rm9		11	87	***	18.32	6.30				***	66.09	18.86						
actcag_rm9	52	18	79													***	20.19	21.29
accctg_rm6	53	9	30							***	6.33	30.93						

sig[1]: significance levels of the Kruskal - Wallis test ***:0,01 ****:0,005 *****:0,001
mean(a)[2]:mean value of clones with absence of the considered marker
mean(b)[2]:mean value of clones with presence of the considered marker
CG[3]: number of the cosegregation group in Hoarau et al (2001)
n(a)4: number of clones with marker absent
n(b)4: number of clones with marker present

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1 data from another biparental progeny, derived from two present-day cultivars R570 and M 695/69, under study in Mauritius island